VERIFICATION OF A TRANSLATION

I, the below-named translator, hereby declare that:

My name and post office address are stated below:

Alessia Sacchi, Via Leonardo da Vinci, 13/A – 22070 Lurago Marinone (CO), Italy.

I am knowledgeable in the Italian and English languages, and I believe the attached English translation of the Italian-language text entitled: "Microemulsioni di retinoidi e composizioni farmaceutiche che le contengono" *("Microemulsions of retinoids and pharmaceutical compositions containing them")* is a true and complete translation of said text.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Alessia Sacchi Date: June 3, 2009

MINISTRY OF PRODUCTIVE ACTIVITIES
GENERAL DIRECTORATE OF PRODUCTIVE DEVELOPMENT AND COMPETITION
ITALIAN PATENT AND TRADEMARK OFFICE
G2 DEPARTMENT

One 10.33 € revenue stamp and seal of the Ministry of Productive Activities Central Patent Office.

Certification of copy of documents relating to a Patent for:
INDUSTRIAL INVENTION No. MI2003A 002019

It is hereby declared that the enclosed copy corresponds to the documents as originally filed with the patent application whose data are as from the enclosed filing certificate

Rome, 15 November 2004

The Public Official
Giampietro Carlotto
(Signature)

WHITE, RED AND GREEN RIBBON AND ORANGE SEAL OF THE MINISTRY OF INDUSTRY, TRADE AND HANDICRAFT.

TO THE MINISTRY OF
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CENTRAL PATENT OFFICE - ROME

Space to be covered
with stamps
FORM A

APPLICATION FOR INDUSTRIAL INVENTION,
FILING MISSING DOCUMENTS, ADVANCED
AVAILABILITY TO THE PUBLIC

A. APPLICANT(S)

J.N.

1)NAME **FIDIA FARMACEUTICI S.P.A.** **SP**
SEATING IN **Abano Terme (Padova)** CODE 00204260285
2)NAME
SEATING IN CODE

B. APPLICANT'S REPRESENTATIVE AT THE CENTRAL PATENT OFFICE
SURNAME, NAME:**BIANCHETTI GIUSEPPE et al.** Taxpayer Identification Number:
NAME OF THE OFFICE: **BIANCHETTI BRACCO MINOJA S.r.l.**
STREET: **Via Rossini** N. **8** TOWN: **MILAN** ZIP CODE: **20122** PROV.: **MI**

C. ADDRESSEE'S ELECTIVE DOMICILE:
STREET: N. TOWN: ZIP CODE: PROV:

D. TITLE proposed class(sect/cl/scl):[] group/sub-group:[]
"Microemulsions of retinoids, and pharmaceutical compositions containing them"

ADVANCED ACCESS TO THE PUBLIC: for months (at the most) YES[ ]NO[X]
IF REQUEST: DATE / / / RECORD N. [ ]

E. NAMED INVENTORS:

surname, name	surname, name
1) **Menegatti Enea**	3) **Esposito Elisabetta**
2) **Cortesi Rita**	4) **Bellato Pierangelo**

F. PRIORITY

COUNTRY OR ORGANIZATION	KIND OF PRIORITY	APPL.N.	FILING DATE	ENCL Y/M
1)			/ /	
2)			/ /	

MISSING DOCUMENTS FILED:	FILING DATE	DOCUMENT N.
1)	/ /	
2)	/ /	

G. AUTHORIZED COLLECTING CENTER FOR MICROORGANISMS CULTURE

H. SPECIAL NOTES:

ENCLOSED DOCUMENTS:

	N. COPIES		
DOC. 1)	[2]	[PROV]	N.PAGES [18] abstract and main drawing, description and claims (1 copy compulsory)
DOC. 2)	[1]	[PROV]	N.SHEETS [06] drawing (1 copy compulsory if

OF DRAWING cited in the description)

DOC. 3)	[1]	[FOLLOWS] power of attorney
DOC. 4)	[0]	[FOLLOWS] designation of inventor
DOC. 5)	[0]	[FOLLOW] priority documents with Italian translation
DOC. 6)	[0]	[FOLLOWS] authorization or assignment act
DOC. 7)	[0]	full name of the applicant

MISSING DOCUMENTS FILED:
DATE RECORD N.
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compare single priorities
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8. Payment receipts, Total amount Euros Two hundred ninety-one/80# compulsory

FILLED IN ON 17/10/2003 **SIGNATURE OF THE APPLICANT(S)**
CONTINUES YES/NO [NO] **Bianchetti Giuseppe (signature)**
CERTIFIED COPY OF THE PRESENT ACT IS REQUESTED YES/NO [YES]

PROVINCIAL OFFICE IND. TRADE HAND. OF MILAN CODE [15]
FILING CERTIFICATE: APPLICATION NUMBER **MI2003A 002019** rec. A
The year **TWO THOUSAND AND THREE** this **SEVENTEENTH** day of the month of **OCTOBER** the above mentioned applicant(s) has(have) produced to me the undersigned the present application, consisting of N. [**01**] additional sheets for the granting of the overmentioned patent.

I. VARIOUS NOTES OF THE ATTESTING OFFICER THE REPRESENTATIVE, EVEN THOUGH INFORMED OF THE CONTENT OF COMMUNICATION NO. 423 OF 03/01/2001, FILES THE DOCUMENT ASKING FOR AN EXTENSION OF TIME FOR SUBMITTING THE POWER OF ATTORNEY

THE PETITIONER **SEAL OF THE OFFICE** **THE ATTESTING OFFICER**
(signature) *Seal of the Ministry of Productive Activities One € 10.33 revenue stamp.* **M. CORTONESI**
(signature)

ADDITIONAL SHEET TO FORM A

ADDITIONAL SHEET N. [01] OF TOTAL SHEETS [01]
APPLICATION N.**MI 2003A 002019** REG.A

A. APPLICANT(S)

		N.G.
[ ]NAME SEATING IN	CODE	[ ]
[ ]NAME SEATING IN	CODE	[ ]
[ ]NAME SEATING IN	CODE	[ ]

E. NAMED INVENTORS:

	surname, name	surname, name
[05]	**Gennari Giovanni**	[ ]
[06]		[ ]
[07]		[ ]
[08]		[ ]
[09]		[ ]

F. PRIORITY

COUNTRY OR ORGANIZATION	KIND OF PRIORITY	APPL.N.	FILING DATE	ENCL Y/M
[ ]			/ /	
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MISSING DOCUMENTS FILED:

FILING DATE	DOCUMENT N.
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PETITIONER'S SIGNATURE: **Bianchetti Giuseppe**
(signature)

SPACE RESERVED TO THE CENTRAL PATENT OFFICE

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ABSTRACT OF THE INVENTION WITH MAIN DRAWING, DESCRIPTION AND CLAIMS

Application N. **MI2003A 002019** Filing date **17/10/2003**
Patent N. Granting date / /

D. TITLE

"Microemulsions of retinoids, and pharmaceutical compositions containing them"

L. ABSTRACT

Disclosed are water-in-oil (W/O) microemulsions containing as active ingredient a retinoid, a phospholipid emulsifier, and possibly hyaluronic acid or salts thereof.

M. DRAWING

7094 Description of the patent for industrial invention of title:

FM/mc **"MICROEMULSIONS OF RETINOIDS, AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM"**

In the name of: **FIDIA FARMACEUTICI S.p.A.**

Seating in: Abano Terme (Padova)

* * *

This invention relates to water-in-oil (W/O) microemulsions containing a retinoid as active ingredient, a phospholipid emulsifier, and possibly hyaluronic acid or salts thereof.

BACKGROUND TO THE INVENTION

Retinoids are defined as a series of compounds which are natural derivatives or synthetic analogues of vitamin A. The role of vitamin A as an essential nutrient was recognised as early as 1913, since which time a great deal of research has been conducted on the product. Wolbach and Howe (J. Exp. Med. 42: 753,1925) first described the histopathological epithelial variations caused by vitamin A deficiency in 1925. This led to the identification of retinol and other natural analogues which began to be used, on a purely empirical basis, as chemopreventive agents of neoplastic transformation.

The role of retinoids in oncological chemoprevention was endorsed by the publication of various epidemiological studies which demonstrated that a regular vitamin A intake was significantly correlated with a lower incidence of tumours, especially lung cancer (Zeigler R.G. et al., Cancer Causes and Control 7: 157-177, 1996; Krishnan K. et al., Primary Care 25: 361-382, 1998).

Only some of the over 4000 retinoids tested to date have a sufficiently favourable therapeutic efficacy/toxicity ratio to allow their clinical use. The relatively recent discovery of nuclear receptors for retinoic acid (which belong to two distinct types, called RAR and RXR) has considerably improved knowledge of their action mechanisms.

Numerous clinical trials have been conducted with retinoids, many of them on skin diseases such as lichen planus and leucoplakia which, due to the high frequency of neoplastic transformation, are classed as pre-cancerous lesions (Hong W et al., N. Engl. J. Med 315: 1501-1505,1986; Lippman S.M et al., N. Engl. J. Med 328: 15-20,1993).

At present, the internationally recognised clinical use of the retinoids relates to the treatment of acute promyelocytic leukaemia and skin diseases with a hyperproliferative component such as acne and psoriasis.

Fenretinide (4-hydroxyphenyl retinamide) is a semi-synthetic retinoid which was developed as a chemoprotective agent (Costa A. et al., Ann. N.Y. Acad. Sci. 768: 148-162,1995; Pienta K.J. et al., Am. J. Clin. Oncol. 20: 36-39,1997).

Unlike other natural retinoids such as all-trans, 13-cis- and 9-cis-retinoic acid, fenretinide does not induce any systemic catabolism which could interfere with the long-term maintenance of pharmacologically useful plasma concentrations. This characteristic, combined with the low toxicity of the product and its ability to inhibit some phenomena associated with carcinogenesis, provides the rationale for the development of fenretinide as a chemoprotective agent in neoplastic disorders such breast, prostate and bladder cancer.

Other phase II trials, conducted on a limited number of subjects, have evaluated the effect of fenretinide on patients suffering from prostate cancer (Pienta K.J. et al., Am. J. Clin. Oncol. 20: 36-39, 1987), melanoma (Modiano M.R. et al., Invest. New Drugs 8: 317-319, 1990) and myelodysplastic syndromes (Garewal H.S. et al., Leukemia Res. 13: 339-343, 1989). However, the results of these studies were rather disappointing, whereas chemoprevention studies conducted on patients suffering from leucoplakia or lichen planus (dermatological lesions which often present neoplastic degeneration) have given promising results (Tradati N. et al., Canc. Letters 76: 109-111, 1994).

In these cases the patients were treated topically, with the result that the fenretinide concentrations reached in the lesion were probably similar to, if not higher than those which have proved active *in vitro*.

At present, topical formulations of retinoids are mainly presented in the form of creams with a fatty base or gels. DE 19946184 describes emulsions of retinoids characterised by a continuous aqueous phase, a mainly non-crystalline viscous oily phase, and a mixture of emulsifiers. Microemulsions of active ingredients which are poorly soluble in water and can be administered by the parenteral, topical or oral route are described in WO 99/56727, EP 211258 and EP 760237.

Topical formulations of retinoids in the form of microemulsions (macroscopically monophasic dispersed systems constituted by at least three components, such as an oily phase, an aqueous phase and a surfactant) are not available. The main chemico-physical properties of microemulsions which characterise their particular structure are transparency, isotropy and thermodynamic stability. As a result of these characteristics, microemulsions are of considerable interest to the pharmaceutical industry. In fact:

(a) the particular microstructure of microemulsions enables molecules with different chemico-physical characteristics to be solubilised;

(b) the transparency of the system makes it possible to check that the active ingredients are completely solubilised;

(c) thermodynamic stability entails major advantages, as the systems obtained are stable for long periods of time.

A further advantage of the topical use of microemulsions is the possibility of increasing the rate of penetration of the active ingredients through the stratum corneum.

Drug release is known to be much faster when gel microemulsions are used rather than conventional formulations (Martini M. et al., J. Pharm. Belg. 39, 348-

354, 1984; Ziegenmeyers J. et al., Acta Pharm. Technol. 26, 273-275, 1980; Ziegenmeyers J. et al., Deuxième Congrès International de Technologie Pharmaceutique 3, pp. 235-238, 1980).

Phospholipids have been used as emulsifying agents to stabilise microemulsions: phosphatidylcholines in an organic solvent (50-250 mM) form small inverse micelles which, on the addition of water, undergo one-dimensional growth, until the formation of a kind of three-dimensional network consisting of a tangle of long, flexible cylindrical structures (Luisi et al., Colloid Polym. Sci. 268, 356-374, 1990).

The presence of water causes a drastic increase in viscosity, leading to the formation of a gelified transparent system, the viscosity of which depends on the content of the aqueous phase. The water content, which is consequently a very important factor in the formation of these particular microemulsions, is expressed by the ratio between the water concentration and the molar lecithin concentration:

[W]/[lec] = [molar water concentration] / [molar lecithin concentration]

The maximum value of [W]/[lec] for each microemulsion depends on the type of organic phase used and the lecithin concentration. The maximum viscosity of lecithin-based gel microemulsions is usually obtained after the addition of less than 10 molecules of water per molecule of lecithin, namely at values of [W]/[lec] <10.

Gel microemulsions based on soy phosphatidylcholine possess all the characteristics of transparency, thermodynamic stability and isotropy typical of microemulsions (Scartazzini R. et al., J. Phys. Chem. 92, 829-833,1988; Luisi et al., Colloid Polym. Sci. 268, 356-374,1990; Lawrence et al., Advanced Drug Delivery Reviews 45, 89-121, 2000).

Phosphatidylcholine is a natural surfactant, and is highly biocompatible (Dreher et al., Skin Pharmacology 9, 124-129,1996).

Hyaluronic acid (HA) is a heteropolysaccharide composed of alternate residues of D-glucuronic acid and N-acetyl-D-glucosamine. It is a linear-chain polymer with a molecular weight ranging between 50,000 and 13 x 10^6 Da, depending on the source from which it is obtained and the preparation methods used. It is found in nature in pericellular gels, in the ground substance of the connective tissue of vertebrates (of which it is one of the main components), and in synovial (joint) fluid, vitreous humour and the umbilical cord.

HA plays an important role in biological organisms, as a mechanical support for the cells of many tissues such as skin, tendons, muscles and cartilage.

It is the main component of extracellular matrix, and also performs other functions such as tissue moisturising and cell lubrication, migration and differentiation.

In view of its properties of bio- and mucoadhesion and its tissue compatibility characteristics, hyaluronic acid and its salts, in particular sodium, potassium, magnesium and calcium salts, possibly suitably fractionated and/or derivatised, have been proposed as systems for the release of drugs and the preparation of surgical aids, implants, prostheses and the like.

DESCRIPTION OF THE INVENTION

It has now been found that retinoids can be advantageously formulated in water-in-oil (W/O) microemulsions using phospholipid emulsifiers, in particular soy phosphatidylcholine and soy lecithin, possibly with the addition of hyaluronic acid or salts and fractions thereof.

The microemulsions of the invention provide better bioavailability than conventional formulations.

The oily phase is preferably constituted by alkyl esters of C_{10}-C_{22} fatty acids.

Isopropyl palmitate is particularly preferred.

Retinoids which can be conveniently formulated in the microemulsions according to the invention include isotretinoin (13-cis-retinoic acid), tazarotene and, in particular, fenretinide.

It has also surprisingly been found that the addition of hyaluronic acid (HA), possibly salified, and in particular sodium hyaluronate or HA derivatives, to microemulsions further increases the bioavailability of the active ingredient.

The HA used in the present invention may derive from any source, such as extraction from rooster combs (EP 0138572), fermentation (EP 0716688), or technological means (Italian patent application no. PD94A000042), and have a molecular weight of between 400 and $3x10^6$Da, in particular between 400 and $1x\ 10^6$Da, and even more particularly between 400 and 200,000 Da.

The HA derivatives which can be used are listed below:

1) HA salified with organic and/or inorganic bases having a molecular weight of 50-730KDa (EP0138572) or a high molecular weight (750-1230 KDa, EP 535200);

2) Hyaff: HA esters with alcohols of the aliphatic, araliphatic, cycloaliphatic, aromatic, cyclic and heterocyclic series, with an esterification percentage which may vary, depending on the type and length of the alcohol used (EP 216453);

3) Hyadd: HA amides with amines of the aliphatic, araliphatic, cycloaliphatic, aromatic, cyclic and heterocyclic series (EP 1095064);

4) O-sulphated HA derivatives up to the 4th degree of sulphation (EP 0702699);

5) ACP: inner esters of HA (EP 0341745).

The fraction known as Hyalastine, a fraction of hyaluronic acid with molecular weights of between 50 and 200 kDa, is preferred.

Hyaluronic acid and its salts act as viscosity agents, and guarantee improved characteristics in terms of stability and bioavailability.

The microemulsions according to the invention may also contain antioxidants and preservatives such as α-tocopherol, alkyl parabens, and other excipients of conventional use.

The weight percentage of active ingredient can range between 0.01% and 0.5%, preferably between 0.05 and 0.15%, while the weight percentage of lecithin or phosphatidylcholine is typically between approx. 10% and approx. 15%. The aqueous phase typically constitutes approx. 0.5 to 2% in weight of the total microemulsions. Sodium hyaluronate can be added as viscosity agent in percentages of between 0.001 and 0.01% in weight.

The microemulsions according to the invention can be prepared by a process which comprises the addition of a solution of phospholipid emulsifier in the oily phase to the retinoid solution in the same oily phase, and subsequent addition of an aqueous solution possibly containing hyaluronic acid, its salts or derivatives, preservatives, EDTA and other ingredients.

EXAMPLES 1-3: Fenretinide microemulsions

Preparation method

α-tocopherol is solubilised in a small volume of isopropyl palmitate (IPP). Soy phosphatidylcholine is solubilised in the remaining volume of isopropyl palmitate at 70°C under stirring until a clear, transparent solution is obtained.

Propyl paraben is then added, and stirring is continued until solubilisation is complete.

The solution is cooled, the solution of α-tocopherol in isopropyl palmitate is added, and the resulting solution is mixed under gentle stirring.

The active ingredient is solubilised in the resulting solution.

Methyl paraben is solubilised in purified water at 80°C to prepare the aqueous phase. The solution is cooled at room temperature, and tetrasodium EDTA and hyaluronic acid sodium salt are solubilised under stirring.

The aqueous solution is added to the isopropyl palmitate oily solution, and the resulting system which is initially turbid, is maintained under stirring until a clear, transparent emulsion of acquired viscosity is obtained.

Qualitative and quantitative compositions

The compositions of the microemulsions obtained by the method described above, which have different W]/[lec] ratios depending on whether they contain sodium hyaluronate, are reported below:

EXAMPLE 1 - IPP2 Hyal ([W]/[lec]: 2).

Constituent	**% w/w**	**Function**
Fenretinide	0.05 – 0.10 – 0.15	Active ingredient
Phosphatidylcholine	14.756	Surfactant
Isopropyl palmitate	q.s. for 100	Oily phase
Purified water	0.7644	Aqueous phase
Hyaluronic acid sodium salt (Hyalastine fraction)	0.0072	Viscosity agent
α-Tocopherol	0.10	Antioxidant

EXAMPLE 2 - IPP3C Hyal ([W]/[lec]: 3).

Constituent	**% w/w**	**Function**
Fenretinide	0.05 – 0.10 – 0.15	Active ingredient
Phosphatidylcholine	14.567 – 14.560 – 14.553	Surfactant
Isopropyl palmitate	q.s. for 100	Oily phase
Purified water	1.1107 – 1.1102 – 1.1096	Aqueous phase
Hyaluronic acid sodium salt (Hyalastine fraction)	0.0108	Viscosity agent
α-Tocopherol	0.10	Antioxidant
Methyl paraben	0.00162	Preservative
Propyl paraben	0.000215	Preservative
Tetrasodium EDTA	0.00108	Complexing agent

EXAMPLE 3 - IPP3C' Hyal ([W]/[lec]: 3).

Constituent	% w/w	Function
Fenretinide	0.05 – 0.10 – 0.15	Active ingredient
Phosphatidylcholine	14.567 – 14.560 – 14.553	Surfactant
Isopropyl palmitate	q.s. for 100	Oily phase
Purified water	1.109 – 1.108 – 1.1096	Aqueous phase
Hyaluronic acid sodium salt (Hyalastine fraction)	0.0108	Viscosity agent
α-Tocopherol	0.10	Antioxidant
Methyl paraben	0.00216	Preservative
Propyl paraben	0.01684	Preservative
Tetrasodium EDTA	0.00108	Complexing agent

EXAMPLE 4: Rheological characterisation

Dynamic viscosity measurements were performed to characterise the gel microemulsions produced; in particular, viscosity measurements were conducted by applying increasing shear rate values, from which the viscosity values at a shear rate of a 70 s^{-1} were extrapolated (measurements conducted at 25°C).

Figure 1 shows the viscosity trend of gel microemulsions based on isopropyl palmitate (IPP) at an active ingredient (fenretinide) concentration of 0.05% w/w, depending on the water content ([W]/[lec] equal to 1, 2 and 3).

The viscosity of IPP1 ([W]/[lec] = 1) and IPP2 ([W]/[lec] = 2) gel microemulsions is considered too low; however, IPP3 gel microemulsions (those with a water content expressed by a [W]/[lec] ratio of 3) are considered to have good viscosity and consistency characteristics for topical application.

Figure 2 shows the viscosity trend of gel microemulsions based on isopropyl palmitate (IPP) which have the same ratio [W]/[lec] (= 3) and concentration as the drug fenretinide (0.05%), but a different qualitative/quantitative composition, and contain hyaluronic acid sodium salt

(Hyalastine fraction) as viscosity agent in order to obtain the viscosity and consistency characteristics considered ideal for the intended uses.

Figure 3 shows the viscosity trend of the microemulsion classed as prototype IPP3C', according to the fenretinide concentration (4HPR), in which slightly inferior viscosity and consistency characteristics are observed as the dose of fenretinide increases.

EXAMPLE 5: Diffusion kinetics of fenretinide

An *in vitro* study was conducted to compare the diffusion or permeation (through a membrane) kinetics of fenretinide in the microemulsions according to the invention with fenretinide in conventional formulations, such as the "ointment base containing cetomacrogol" and "fatty cream base containing cetomacrogol" described in the Italian Official Pharmacopoeia (F.U.), current edition.

"Franz diffusion cells" associated with a cellulose membrane were used to study the diffusion kinetics of fenretinide in the different formulations.

The cell consists of two glass sections, one above the other. The inner diameter of the cell is 1 cm (equal to an area of 0.78 cm^2).

The formulation containing fenretinide was placed in the top section, while the bottom section was filled with the receptor phase constituted by methanol, and maintained under constant stirring by means of a magnetic "follower" fitted at the base of the cell.

The membrane, previously moistened with the receptor phase, was placed between the two sections. The two sections of the cell were assembled, taking care to avoid the formation of air bubbles. All the experiments were carried out under the same conditions: shaded from the light, at the temperature of 25°C, the bottom section being thermostated at 32°C, and 4 cells being used in parallel.

Samples of the receptor phase were taken at pre-set intervals (from 30 minutes to 8 hours) using a syringe with a flexible teflon tube; each sample was then replaced with an equal volume of receptor phase.

The samples were analysed for their fenretinide content using reverse-phase HPLC chromatography. The total quantities of fenretinide released per area unit ($\mu g /cm^2$) were calculated from the results of the chromatographic analysis.

The tests were repeated in quadruplicate for each formulation. The mean values were then calculated and shown in the graph as a function of time, expressed in hours.

The test points were interpolated with a linear regression calculation, obtaining lines of the type "y=mx + q". Angular coefficient "m", which represents the slope of the line, corresponds to test flow "Js".

It is known that Js = C x Jn, where C is the concentration of the drug expressed in mg/ml.

As the fenretinide concentration in all the formulations analysed was 1 mg/ml, "Js" is equal to "Jn". "Jn" represents the diffusion coefficient, namely the flow rate at which the drug permeates through the membrane.

Figure 4 shows the diffusion or permeation kinetics of fenretinide (absorption rate) in gel microemulsions IPP3C and IPP3C' (without hyaluronic acid sodium salt, Hyalastine fraction) by comparison with fenretinide in conventional formulations ("ointment base containing cetomacrogol" and "fatty cream base containing cetomacrogol"); the angular coefficients of the lines obtained represent the values of diffusion coefficient "Jn".

Figure 5 shows the diffusion or permeation kinetics of fenretinide (absorption rate) in the same gel microemulsions containing hyaluronic acid sodium salt (Hyalastine fraction) by comparison with fenretinide in conventional formulations ("ointment base containing cetomacrogol" and "fatty cream base containing cetomacrogol").

Figure 6 shows the diffusion or permeation kinetics of fenretinide (absorption rate) in gel microemulsions IPP3C and IPP3C' with or without hyaluronic acid sodium salt (Hyalastine fraction).

The diffusion (or permeation or absorption) coefficients "Jn" of fenretinide carried in gel microemulsions type IPP3 ([W]/[lec] = 3), in the absence and presence of hyaluronic acid sodium salt (Hyalastine fraction), and in conventional formulations, are set out in the table below:

Formulation	**Fenretinide (% w/v)**	**J_n (μg/cm²/h)**	**log J_n**
Ointment base, F.U	0.1	**0.15**	-0.82
W/O cream base, F.U.	0.1	**0.50**	-0.3
IPP 3 C	0.1	**13.43**	1.12
IPP 3 C HYAL	0.1	**18.47**	1.26
IPP 3 C'	0.1	**10.22**	1.00
IPP 3 C' HYAL	0.1	**16.19**	1.20

The following conclusions can be drawn from the test results obtained:

- The kinetics of diffusion or permeation (absorption rate) of fenretinide carried as a gel microemulsion based on phospholipids (soy phosphatidylcholine) are considerably greater than those of the drug when carried in conventional formulations (W/O cream or ointment); the diffusion coefficients "Jn" of fenretinide carried as a gel microemulsion are considerably greater than those obtained with an ointment (approx. 79 times higher) and/or a W/O cream (approx. 24 times higher).
- Surprisingly, the kinetics of diffusion or permeation (absorption rate) of fenretinide carried as a gel microemulsion based on phospholipids (soy phosphatidylcholine) are considerably increased by the presence in the formulation of hyaluronic acid sodium salt (Hyalastine fraction), which therefore has the effect of promoting percutaneous absorption.

CLAIMS

1. Water-in-oil (W/O) microemulsions containing a retinoid and a phospholipid emulsifier as active ingredient.

2. Microemulsions as claimed in claim 1, wherein the phospholipid emulsifier is selected from soy phosphatidylcholine and soy lecithin.

3. Microemulsions as claimed in claim 1 or 2, wherein the oily phase consists of alkyl esters of C_{10}-C_{22} fatty acids.

4. Microemulsions as claimed in claim 3, wherein the oily phase consists of isopropyl palmitate.

5. Microemulsions as claimed in one or more of the preceding claims, wherein the retinoid is selected from isotretinoin (13-cis-retinoic acid), tazarotene and fenretinide.

6. Microemulsions as claimed in claim 5, wherein the retinoid is fenretinide.

7. Microemulsions as claimed in one or more of the preceding claims, also containing sodium hyaluronate.

8. Microemulsions as claimed in one or more of the preceding claims, containing a derivative of hyaluronic acid selected from:

- HA salified with organic and/or inorganic bases with a molecular weight of 50-730 KDa or a high molecular weight (750-1230 KDa);
- esters of HA with alcohols of the aliphatic, araliphatic, cycloaliphatic, aromatic, cyclic and heterocyclic series;
- amides of HA with amines of the aliphatic, araliphatic, cycloaliphatic, aromatic, cyclic and heterocyclic series;
- O-sulphated derivatives of HA up to the 4th degree of sulphation;
- inner esters of HA.

9. Microemulsions as claimed in one or more of the preceding claims, also containing antioxidants and preservatives.

10. Microemulsions as claimed in claim 9, containing α-tocopherol and parabens.

11. Pharmaceutical compositions comprising the microemulsions described in claims 1-10.

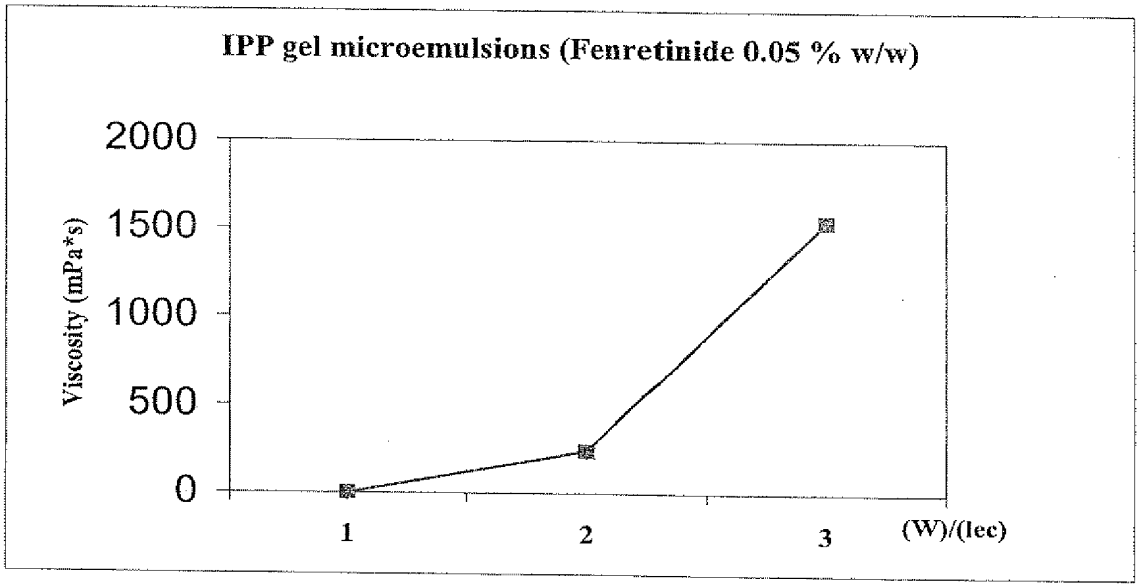
12. Use of the microemulsions described in the claims 1-10 for the preparation of medicinal products with chemoprotective activity.

13. A process for the preparation of the microemulsions claimed in claims 1-10, which involves the addition of a solution of phospholipid emulsifier in the oily phase to the retinoid solution in the same oily phase, or the subsequent addition of an aqueous solution, possibly containing hyaluronic acid, salts or derivatives thereof, preservatives, EDTA and optionally other components.

Milano, 17 October 2003

The Agent
(Bianchetti Giuseppe)
of BIANCHETTI BRACCO MINOJA S.r.l.
(signature)

FIGURE 1



The Agent
(Bianchetti Giuseppe)
of BIANCHETTI BRACCO MINOJA S.r.l.
(signature)

FIGURE 2

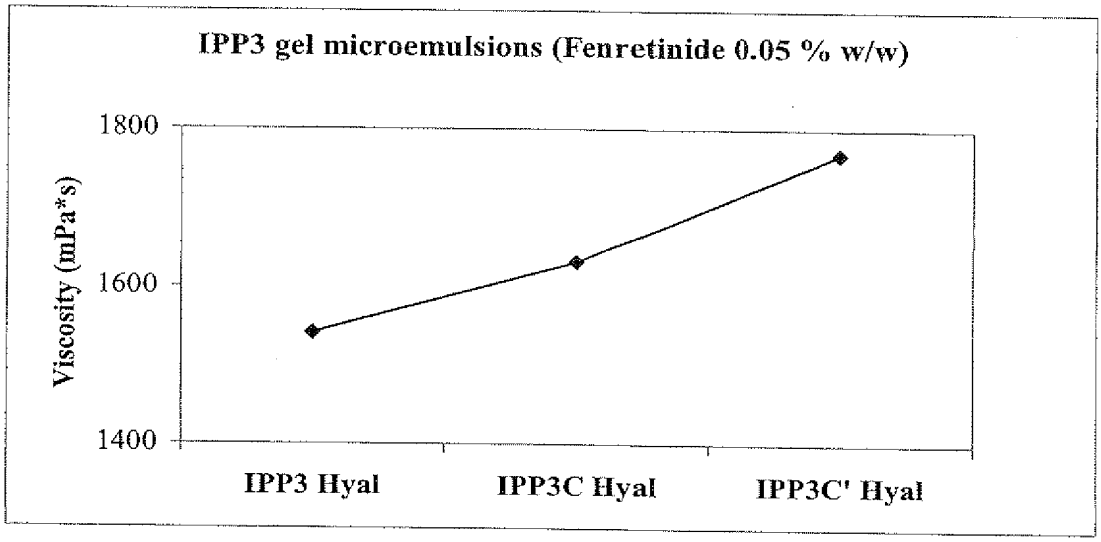

IPP3 gel microemulsions (Fenretinide 0.05 % w/w)
Viscosity (mPa*s)
1800
1600
1400
IPP3 Hyal
IPP3C Hyal
IPP3C' Hyal

FIGURE 3

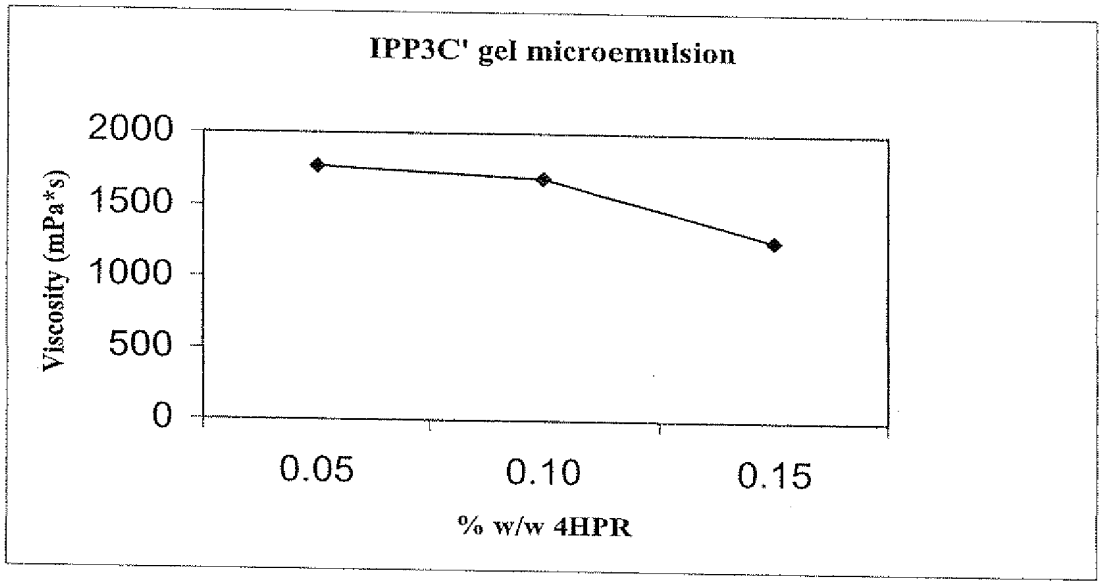


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FIGURE 4

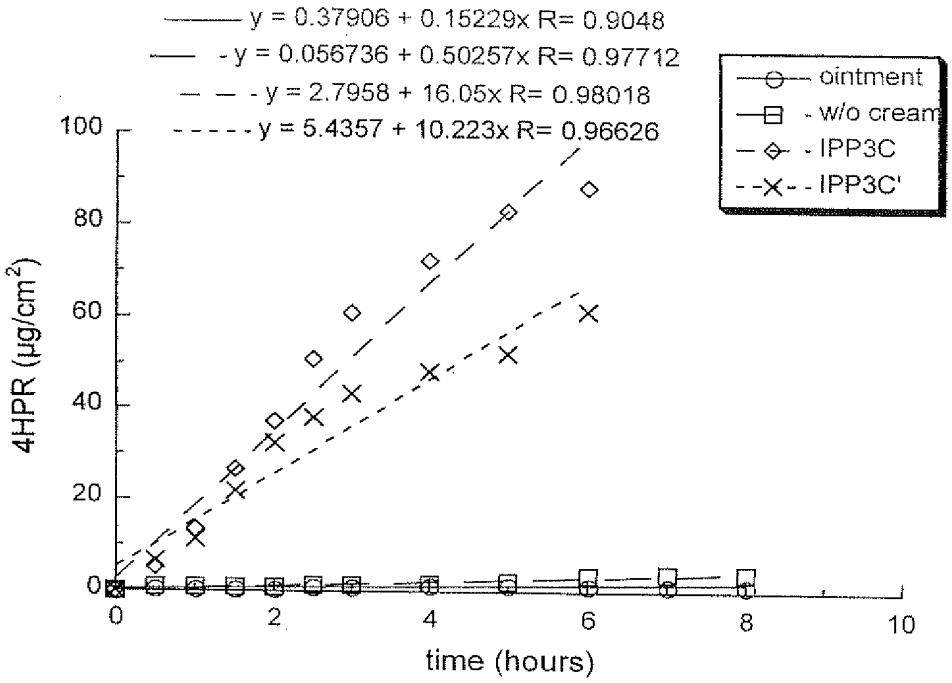

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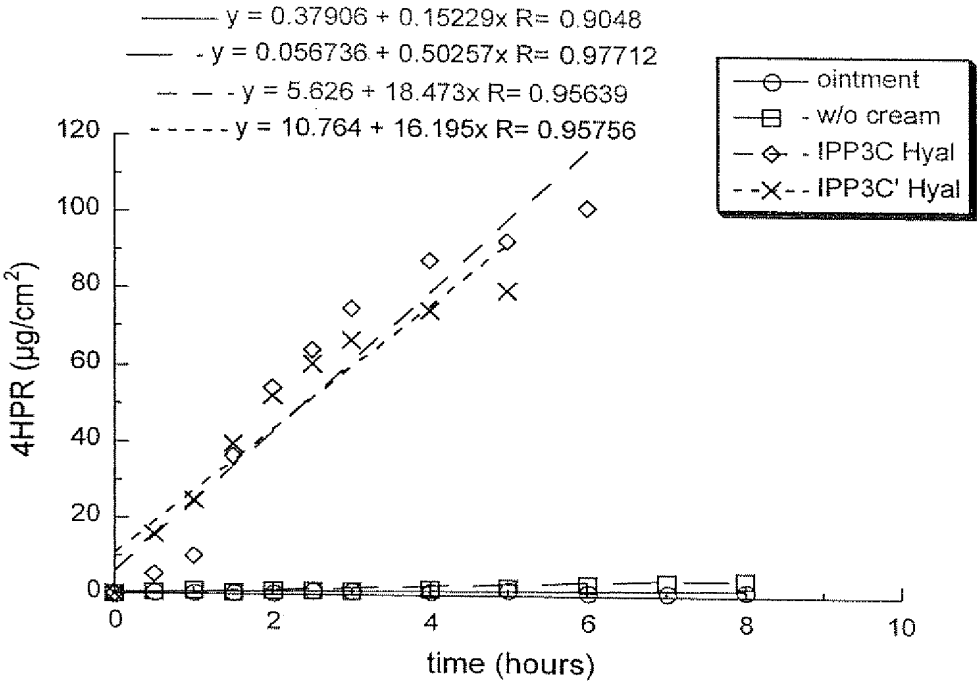
FIGURE 5



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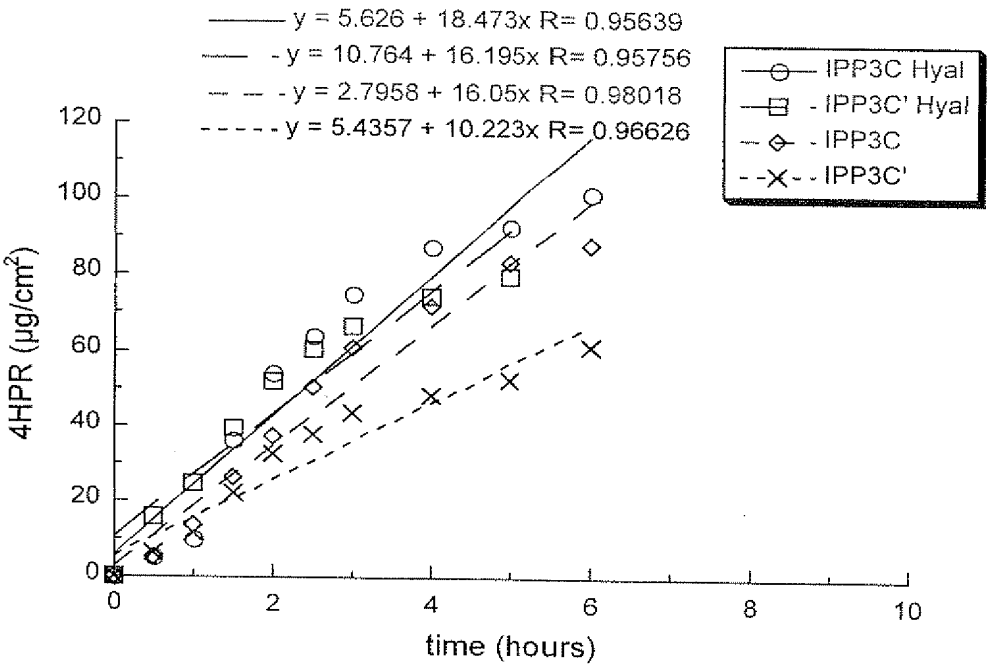
(signature)

FIGURE 6



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(signature)